EVANESCENT WAVE BACKGROUND FLUORESCENCE/ABSORBANCE DETECTION

Continuation-in-part of Ser. No. 06/818/721, Jan. 14, 1986, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to method and apparatus for the detection and measurement of certain optical characteristics of biological culture and fermentation media in a bioreactor or the like, and more particularly, to the measurement of the fluorescence and/or absorbance of a continuous phase in the presence of a discontinuous phase.

2. Description of Related Art

Fluorescence of intracellular NADH or NADPH has been shown to be a good indicator of the metabolic state of cells in culture, as well as serving as an indicator of the concentration of cells in the culture medium. Several papers have attested to the value of this kind of in situ measurement for both microbial and yeast culture applications. See, e.g., W. B. Armiger et al., Analysis and Control of Fed-Batch Fermentations Producing *Escherichia coli* Using Culture Fluorescence, Proceedings Biotech **84**, Washington, D.C. 1984. Apparatus for performing these measurements is available from Bio-Chem Technology, Inc., Malvern, Pa., as the FluoroMeasure TM System.

From an economic point of view, it would be advantageous to use lower cost complex nutrients, such as molasses or corn steep liquor in industrially significant cultures. These, however, introduce additional background fluorescence. If the medium contains a fluorescent component which does not change during the course of the culture, a background correction can be made simply by subtracting the reading at time zero from all subsequent readings. In a case where the fluorescence of the media changes due to use by the cell or in the case where the cells produce a competing fluorescence, it becomes more difficult to correct on-line for changes in background. This can be done for a batch culture by taking serial samples, removing the cells and measuring the fluorescence of the medium. Even more difficult are background corrections in cases of continuous culture or where nutrients are added stepwise during the culture.

For fluorescent media, it would be useful to be able to determine how the fluorescence of the media is changing during the fermentation, such that a measurement can be made to the metabolic state of the cells and their growth rate. What is needed is a means of effecting the separation of the cell fluorescence from that of soluble materials.

Optical sensors for fermentations or tissue culture are capable of giving information on intracellular substances and conditions. Such information would permit a finer control based on actual intracellular information rather than on the existing on-line sensors, temperature, pH, dissolved oxygen, off-gas analysis. It would lead to a better scaleup and commercialization of products derived from recombinant DNA and cell fushion technologies. Optical sensors, at present, work best with media which do not interfere since there is not easy way to correct for changes in optical background.

The early work with optical sensors for following intracellular metabolism dates back to 1957, when Duysen and Amesz observed that the fluorescence of baker's yeast was similar to that of NADH and that the fluorescence of starved yeast could be enhanced by adding ethanol or glucose to the suspension. Later, Harrison and Chance built an instrument capable of measuring culture fluorescence in situ and could monitor aerobic/anaerobic transitions in continuous culture. Using a similar device, Humphrey and coworkers, and others, have shown that a fluorometer placed on a fermentor could measure intracellular NADH changes and might be useful for process control. Zabriskie and Humphrey showed the linear relationship between the logarithm of the fluorescence of the culture and the logarithm of cell concentration. Ristroph et al. studied the relationship between culture fluorescence and the growth of *Candida utilis* in a fed batch fermentation.

These studies have shown that the concentration in intracellular NADH measured by culture fluorescence in a fermentation is a function of the number of cells, the energy level within each cell, and the level of metabolic activity. A mathematical expression which is derived from these studies is:

$$F(t)=[Y_{f/x}(1+m(t))]X(t)+E(t)$$

X(t) is the cell concentration. The term in square brackets is the fluorescence yield, which is made up of an invariant compound $Y_{f/x}$, which is characteristic of the type of organism and a variable component m(t), which changes in response to shifts in the level of metabolic activity. The final term, E(t), with which the present invention is mainly concerned, is the environmental, or background, fluorescence. Obviously, if E(t) fluctuates during the fermentation, then it would be difficult if not possible to derive information about the cells from the measured overall fluorescence. Continuous, or batch fed fermentation or cell cultures only exacerbate the problem. In those techniques, additional variables are introduced without corresponding information as to concentration.

Almost all of the published studies have used synthetic media where E(t) is low, or the corrections for E(t) had to be arrived at empirically, In scaling up fermentations and cell cultures for commercial production, economic factors may dictate use of the natural nutrients, like molasses or fetal calf serum, which have a natural fluorescence and therefore contribute to the background value. When checking some of the assumptions used in correcting for the background, I found indications that the background fluorescence of, for instance, molasses, and the fluoresence of yeast cells do not add linearly. This pointed up the need for a method for continuously measuring the media fluorescence background on-line and in real time, i.e. using a sensor or sensors continuously monitoring the detected variable as the fermentation or culture is being conducted.

This means that, without physically separating the cells from the media, a method was needed which caused the media to fluorescence without, at the same time, causing the cells to fluoresce. In accordance with the present invention, the evanescent wave phenomenon is used to meet this need.

SUMMARY OF THE INVENTION

When a beam of light is totally reflected from a non-mirrored interface between two optically transparent media of different refractive indexes, an evanescent wave phenomenon, such as shown in FIG. **1**, exists. The light beam **11** is totally reflected from this kind of surface, unlike a mirrored surface, and behaves as though it